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A rapid method for the extraction of whole cell proteins from *Candida* species

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Abstract

A rapid method for the extraction of whole cell proteins from yeasts of the genus *Candida* has been developed. Stationary phase cells (10^9) were harvested, washed with phosphate-buffered saline and resuspended in 200 μl of sodium dodecyl sulphate (SDS) sample buffer. The cell suspension was boiled for 20 min and 20 μl were applied directly to a 12.5% polyacrylamide gel. Electrophoresis was performed for 18 h at 75 V, after which time, staining with Coomassie Brilliant Blue R 250 revealed the presence of approximately 30 distinct bands, depending upon the species. This method of extracting whole cell proteins is rapid, easy to perform and has been used with a range of *Candida* species (*viz. C. albicans*, *C. tropicalis*, *C. pseudotropicalis*, *C. parapsilosis*, *C. lusitaniae*, *C. krusei* and *C. glabrata*) obtained from both laboratory stocks and clinical samples. It enables the differentiation of a large number of *Candida* species based upon the protein banding pattern(s) obtained after SDS–polyacrylamide gel electrophoretic analysis.

Keywords: *Candida*; SDS–PAGE; Whole cell protein

1. Introduction

Candida albicans is an opportunistic fungal pathogen that is the principal cause of superficial and systemic candidiasis (Cutler, 1991). Cases of superficial candidiasis [oral candidiasis, vulvovaginal candidiasis (VVC)] are relatively common whereas systemic candidiasis occurs infrequently, mainly in severely immunocompromised patients and can prove fatal. VVC affects approximately 75% of women at some point and is caused predominantly, although not exclusively, by *C. albicans*. Other *Candida* species that have been implicated in this condition include *Candida glabrata* (Redondo-Lopez et al., 1990) and *Candida tropicalis* (Geiger, 1995). Recurrent vulvovaginal candidiasis (RVVC) affects approximately 5% of women and the factors responsible for its' persistence are poorly characterised (Odds, 1993). In many instances of VVC and RVVC, more than one species of yeast may be responsible for infection, so a rapid method for differentiating *Candida* species is required (Odds, 1993).

Several methods have been developed for the identification and differentiation of *Candida* species. Morphotyping (Phongpaichit et al., 1987), resistogram typing (McCreight and Warnock, 1982), karyotyping (Schwartz and Cantor, 1984), restriction endonuclease analysis of genomic DNA (Vazquez et

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al., 1993) and polymerase chain reaction (PCR) (Haynes et al., 1995) have been widely used in recent years for the identification of yeasts. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) has been employed to analyse the constituents of soluble extracts obtained from intact cells and isolated cell walls. Extraction methods have included treatment of cells or isolated cell walls with β-mercaptoethanol and SDS (Chaffin and Stocco, 1983) or using combinations of dithiothreitol, protease, lyticase and chitinase (Ponton and Jones, 1986). Rupture of cells by agitation with glass beads or sonication has allowed the extraction of proteins from *C. albicans* and *Saccharomyces cerevisiae*, however, these methods are time-consuming and require specialized equipment (Catley, 1990). While a number of methods exist for the extraction of proteins from yeast cells, the method employed can affect both the composition and size of moieties present in the extract (Casanova and Chaffin, 1991).

Whole cell protein profiles obtained by SDS–PAGE may be labelled radioactively or with stains such as Coomassie Brilliant Blue and have been used for the differentiation of yeast species (Vancanneyt et al., 1991). Horvath and Riezman (1994) developed and evaluated a rapid method for the extraction of proteins from *S. cerevisiae* for SDS–PAGE and Western blotting. This extraction procedure has the advantage of being rapid and easy to perform and could be used for the routine identification and differentiation of a large number of yeast strains, although it was not equally applicable to all yeasts tested.

A modification of the method of Horvath and Riezman (1994) for the rapid extraction of whole cell proteins from a number of *Candida* species is presented here. These could subsequently be analysed by SDS–PAGE to allow the identification and differentiation of clinically important species.

2. Materials and methods

2.1. Yeast cultures

The following yeasts were used for the extraction of whole cell proteins: *C. albicans* 10231 and 44990 were obtained from the American Type Culture Collection, Rockville, MD, USA. *C. tropicalis* 3097 and 3109, *Candida pseudotropicalis* 3106 and 3234, *Candida parapsilosis* 3207 and 3209, *Candida krusei* 3100 and 3321, and *C. glabrata* 4733 were obtained from the National Collection of Pathogenic Fungi, London, UK.

Ten clinical yeast isolates (five *C. albicans*, three *C. glabrata*, one *Candida lusitaniae* and one *C. krusei*) were obtained from patients with symptoms of vaginitis. Vaginal samples were collected with sterile cotton swabs and inoculated onto CHROMagar plates (Mast Diagnostic, Bootle, UK) and incubated at 37°C for 48 h. All yeast isolates observed on CHROMagar were identified by colony morphology and pigmentation (Odds and Bernaerts, 1994). Identification was confirmed using the API 20C AUX test system (Bio Merieux, Marcy L'Etoile, France) and, in the case of *C. albicans*, by the ability to form germ tubes in foetal calf serum at 37°C and to produce chlamydospores on corn meal agar.

2.2. Culture conditions

Yeast isolates were grown to the stationary phase (approximately 5×10^8/ml) overnight in 50 ml of YEPD liquid medium [2% (w/v) glucose (Sigma, St. Louis, MO, USA), 2% (w/v) Bactopeptone (Oxoid, Basingstoke, UK) and 1% (w/v) Yeast Extract (Oxoid)] in 100 ml conical flasks at 30°C and 200 rpm in an orbital incubator. Cultures were maintained on YEPD agar plates [as above, but solidified with the addition of 2% (w/v) agar (Difco, Detroit, MI, USA)] at 4°C and were sub-cultured every four weeks.

2.3. Whole cell protein extraction

Stationary phase cultures were harvested by centrifugation at 2280 *g* for 5 min in a Beckmann GS-6 centrifuge, washed twice with phosphate-buffered saline (PBS) and 10^9 cells were resuspended in 200 μl of sample buffer [0.06 M Tris–HCl, pH 6.8, 2% (w/v) SDS (Sigma), 5% (v/v) β-mercaptoethanol, 10% (v/v) glycerol, 1 mM phenylmethylsulfonylfluoride (Sigma) and 0.5% (w/v) bromophenol blue (Sigma)]. The suspensions were boiled for 20 min and 20 μl were applied directly to each well of a 12.5% (w/v) polyacrylamide gel.

2.4. SDS–PAGE

One-dimensional SDS–PAGE was performed using a modification of the technique of Hames (1990). Samples were loaded onto a 12.5% (w/v) polyacrylamide gel in a discontinuous buffer system and subjected to electrophoresis at 75 V for 18 h. The reservoir buffer consisted of 0.25 M Tris–HCl, 1.92 M glycine (Sigma) and 1% (w/v) SDS (pH 8.3). The gel was stained with 0.05% (w/v) Coomassie Brilliant Blue R 250 (Sigma) for 4 h and destained in 10% (v/v) acetic acid and 20% (v/v) methanol.

Protein standards used for estimation of molecular weight were: bovine albumin, 66,000 Da; egg albumin, 45,000 Da; glyceraldehyde-3-phosphate dehydrogenase, 36,000 Da; bovine carbonic anhydrase, 29,000 Da; bovine pancreas trypsinogen, 24,000 Da; soybean trypsin inhibitor, 20,000 Da and bovine milk α-lactalbumin, 14,200 Da (Sigma).

3. Results

A rapid method for the extraction of proteins from *S. cerevisiae* was developed by Horvath and Riezman (1994). This involved the suspension of approximately 5×10^7 exponential phase cells in 100 μl of SDS sample buffer, heating to 95°C for 5 min and clarification of the sample by centrifugation at 14,000 *g* for 5 min. Samples (30–50 μl) of the supernatant were then applied to a SDS gel and analysed by PAGE. Application of this technique to *C. albicans* gave very poor results. Increasing the sample cell number from 5×10^7 to 1×10^9 failed to yield sufficient protein to produce a well-defined protein profile using this method, so a number of variations were attempted in order to develop a method that would retain the rapidity and ease of use of the method of Horvath and Riezman (1994) but increase its applicability to a range of *Candida* species.

C. albicans 10231, 44990 and five *C. albicans* isolates obtained from clinical samples were grown to the stationary phase in YEPD medium overnight, harvested by centrifugation, washed with PBS and 1×10^9 cells were resuspended in 200 μl of SDS sample buffer and boiled for 20 min. A sample (20 μl) of this cell suspension was subsequently applied directly to a 12.5% polyacrylamide gel as described. SDS–PAGE analysis revealed the presence of approximately 30 distinct protein bands with molecular weights ranging in size from 63,000 to 12,000 Da (Fig. 1). The protein banding patterns of the five *C. albicans* clinical isolates were identical to those of *C. albicans* 10231 and 44990 (Fig. 1) and distinct from those of other *Candida* species (Fig. 2 Fig. 3 Fig. 4).

The applicability of this technique to a range of *Candida* species was established by extracting whole cell proteins from stationary phase cells of *C. glabrata* 4733 and the three *C. glabrata* clinical isolates. The extraction procedure involved harvesting 10^9 cells and boiling them in SDS sample buffer

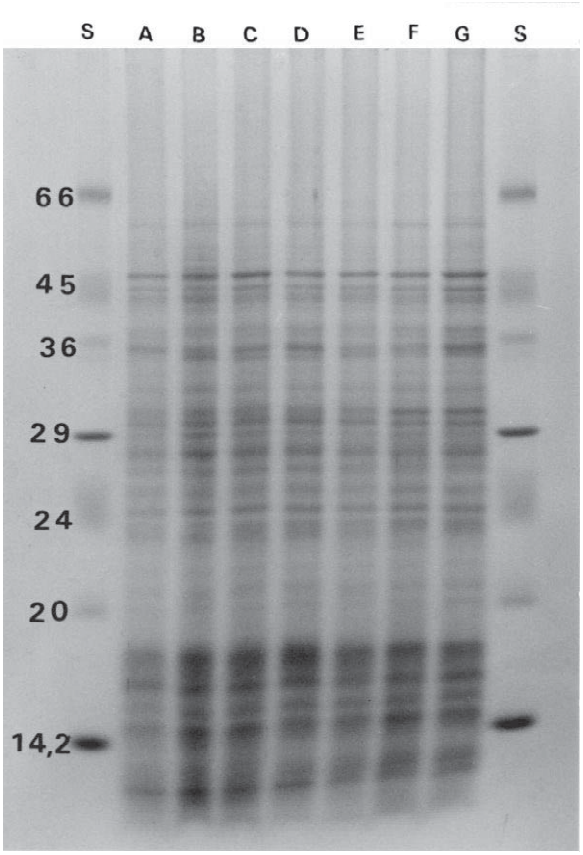

Fig. 1. Protein banding patterns of *Candida albicans* isolates. Proteins were extracted as described from seven *C. albicans* isolates and analysed by SDS–PAGE. The following isolates were used: *C. albicans* 10231 (lane A), *C. albicans* 44990 (lane B) and *C. albicans* clinical isolates (lanes C–G). The migration of the molecular weight standards is shown in lane S ($M_r \times 10^{-3}$).

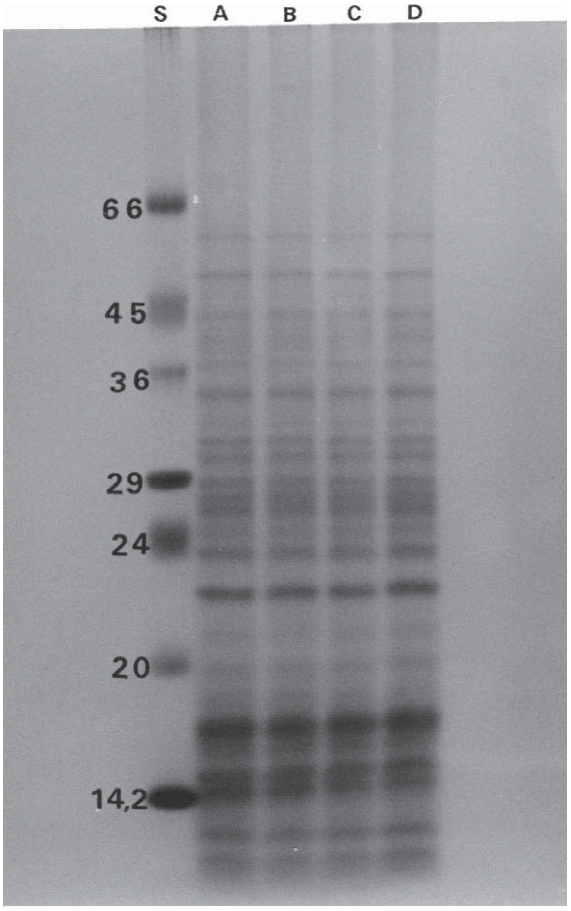

Fig. 2. Protein profiles of *Candida glabrata* isolates. Whole cell proteins were extracted from *C. glabrata* 4733 (lane A) and three *C. glabrata* clinical isolates (lanes B–D) as described. The molecular weight standards are present in lane S ($M_r \times 10^{-3}$).

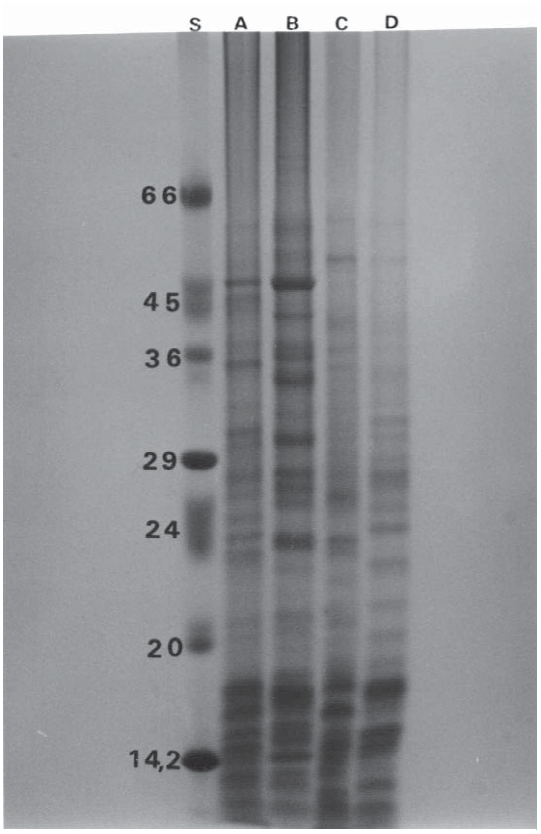

Fig. 3. Protein profiles of clinical isolates of *Candida* species. Proteins were extracted as described and analysed by SDS–PAGE. The following clinical isolates were used: *C. albicans* (lane A), *C. lusitaniae* (lane B), *C. krusei* (lane C) and *C. glabrata* (lane D). The molecular weight standards are in lane S ($M_r \times 10^{-3}$).

for 20 min prior to loading onto a 12.5% polyacrylamide gel. The gel showed a high degree of similarity between the protein banding patterns of the four *C. glabrata* isolates (Fig. 2), but the banding patterns were distinct from those of the *C. albicans* isolates (Fig. 1) and the other *Candida* species (Figs. 3 and 4).

This method of whole cell protein extraction was also used to distinguish between the four *Candida* species obtained from clinical specimens. Proteins were extracted as described. SDS–PAGE revealed the clearly distinguishable protein banding patterns of the different clinical isolates (Fig. 3). Whole cell proteins were also extracted using this method from two isolates each of *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. pseudotropicalis* and *C. krusei* and, again, there are clear differences between the protein banding patterns obtained after SDS–PAGE of each species and a high degree of similarity between those profiles obtained from the same species (Fig. 4).

4. Discussion

Polyacrylamide gel electrophoresis plays a major role in the experimental analysis of proteins and protein mixtures. One-dimensional polyacrylamide gel electrophoresis is still the most widespread form of the technique (Hames, 1990) and has been used to separate proteins from cell extracts after treatment

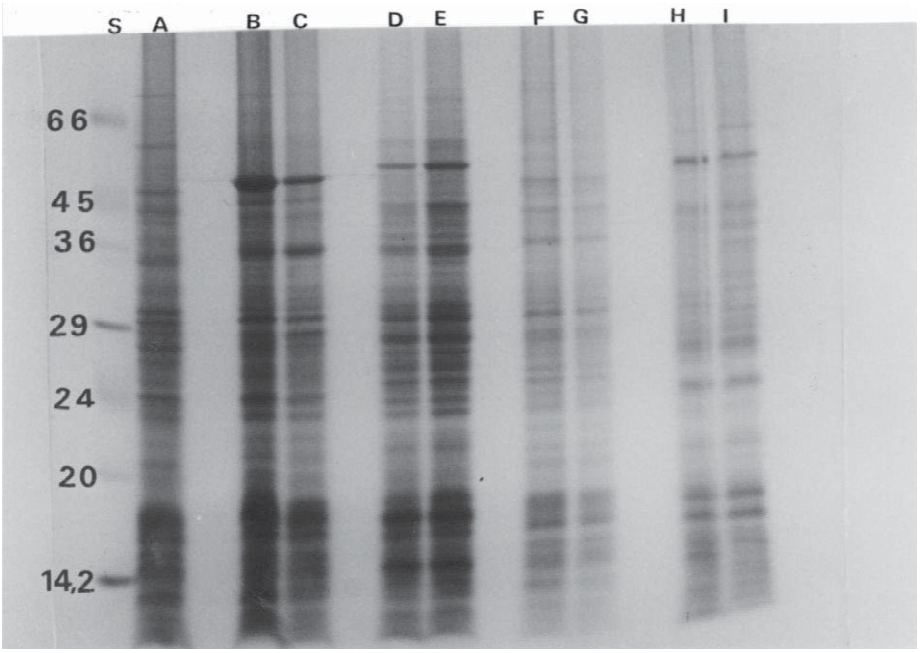

Fig. 4. Protein profiles of *Candida* species. *C. albicans* 10231 (lane A), *C. tropicalis* 3097 and 3109 (lanes B and C, respectively), *C. parapsilosis* 3106 and 3234 (lanes D and E), *C. pseudotropicalis* 3207 and 3209 (lanes F and G) and *C. krusei* 3100 and 3321 (lanes H and I). The migration of the molecular weight standards is shown in lane S ($M_r \times 10^{-3}$).

with SDS and β-mercaptoethanol. Protein bands can be visualized radioactively or by staining with Coomassie Brilliant Blue and protein profiles from different yeast species can be compared in order to facilitate identification (Vancanneyt et al., 1991).

In this study, a simple method for the extraction of whole cell proteins from a range of *Candida* species has been developed. The basic differences between this method and that described by Horvath and Riezman (1994) for *S. cerevisiae* is that, after boiling stationary phase cells (rather than exponential phase cells) in SDS sample buffer for 20 min (rather than 5 min), 20 μl of cell suspension (rather than the supernatant) are applied directly to the polyacrylamide gel. This facilitates the production of a well-defined protein banding pattern, which can be used to identify and differentiate *Candida* species. In contrast, loading 20 μl of SDS sample buffer containing cell supernatant failed to give a protein banding pattern. Boiling in SDS sample buffer is insufficient to release enough protein from cells to allow the production of a distinct banding pattern after SDS–PAGE. It appears that the majority of proteins are extracted from the cells during electrophoresis. The more rigorous extraction procedure required to obtain proteins from *Candida* species may be partly explained by the presence of the strong, multi-layered cell wall retarding cell lysis (Cassone, 1989).

It has been demonstrated here that the protein banding patterns of *C. albicans* 10231 and 44990 are identical to those of five *C. albicans* isolates obtained from women with symptoms of VVC and distinct from the banding patterns of a number of *C. glabrata* isolates (Fig. 2). Similarly, the banding patterns of laboratory isolates and clinical isolates of *C. glabrata* show a high degree of homology. When samples of a number of different *Candida* species are run on the same gel, clear differences that aid species differentiation are visible (Figs. 3 and 4).

This modification of the method of Horvath and Riezman (1994) allows the rapid extraction of proteins from a range of *Candida* species. The procedure is faster and less labour intensive than methods that depend upon the extraction of proteins from cells by agitation with glass beads or by

sonication and it gives sufficient protein from 10^9 stationary phase cells to allow the production of well-defined protein profiles by SDS–PAGE. This method enables the identification and differentiation of *Candida* species from clinical samples by comparing the profiles with those of known *Candida* species from culture stocks. Using this method, whole cell proteins can be extracted from between ten and 15 stationary phase yeast cultures, separated by SDS–PAGE and the proteins can be visualized by staining. While other methods of yeast identification are faster (e.g. PCR-based techniques), the method described here is less labour intensive than conventional methods of protein extraction and offers the possibility of examining a large number of isolates simultaneously.

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